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Applicant: Gardner et al. | Confirmation No. 4887

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Docket No. UTSD:1510 | Examiner: Nashed, Nashaat T.

Title: NMR Detection of Foreign PAS Domain Ligands

DECLARATION UNDER 37CFR1.132

I, Professor Stephen R. Sprang, declare and state as follows:

1. I am a Professor in the Department of Biochemistry at the University of Texas Southwestern Medical School. The Board of Regents of the University of Texas System is the assignee of this patent application. I have authored numerous scientific papers in the field of protein regulation, and I am familiar with this patent application. A copy of my curriculum vitae is attached.

2. PAS domains are one of the most well-studied and documented protein domains, subject to hundreds of scholarly publications. The Specification teaches and exemplifies the claimed methods with a wide variety of suitable PAS domains including PAS kinase PAS A, NPAS2 PAS A, HIF2α PAS B, HIF1α PASB, ARNT PAS B and HERG N-terminal PAS domain (p.6, lines 3-5; p.14, line 8 - p.21, line 10). Those skilled in the art recognize in the Specification a description of the claimed method of detecting binding of a PAS domain with a foreign small molecule ligand that binds into the core of these domains. The practitioner does not require a description of the atomic structure or amino acid sequence of every or any targeted PAS domain to practice the invention.

3. While there is sequence variability across disparate PAS domains, those skilled in the art appreciate what is, and what is not a PAS domain, recognize the term PAS domain as definite, and recognize its metes and bounds. Indeed, the presence of PAS domains within a protein sequence can be identified using several computational methods that are publically available.

A foreign ligand of a PAS domain is distinct from a natural ligand naturally associated with the PAS domain in its host. This is consistent with the Specification which defines the foreign ligand as "not a natural ligand of the PAS domain" (e.g. p.4, lines 22-23) and "foreign to the [PAS domain] host." (e.g. p.5, line 24). One skilled in the art can discern what is, and what is not, a foreign ligand of a PAS domain, recognizes the term foreign ligand of a PAS domain as definite, and recognizes its metes and bounds.

The claims require that the recited PAS domain comprises a hydrophobic core that has no

NMR-apparent a priori formed ligand cavity. This clause is literally self-explanatory to one skilled in the art, meaning literally that the core has no NMR-apparent a priori ("before experience") formed ligand cavity. This clause distinguishes and excludes those cores that have an NMR-apparent a priori formed ligand cavity. One skilled in the art can discern what is, and what is not, an NMR-apparent a priori formed ligand cavity of a PAS domain hydrophobic core, recognizes the clause as definite, and recognizes its metes and bounds.

The claims require comparing the first NMR spectrum with a second NMR spectrum of the PAS domain in the absence of the ligand to infer the presence of the ligand specifically bound within the hydrophobic core of the PAS domain. This step is literally self-explanatory to one skilled in the art, requiring literally that the practitioner compare the first NMR spectrum (in the presence of the ligand) with the second NMR spectrum of the PAS domain (in the absence of the ligand) to infer the presence of the ligand specifically bound within the hydrophobic core of the PAS domain. The step literally requires that the practitioner compare the NMR spectra to infer the presence of specific ligand binding. Details and examples of how the practitioner infers the presence of specific ligand binding from compared NMR spectra are provided, *inter alia*, at p.13, line 29 - p.21, line 10. In the context of the disclosure, one skilled in the art can readily discern what it means to infer the presence of the ligand specifically bound, recognizes the phrase as definite, and recognizes its metes and bounds.

4. As explained in the Specification some members of the PAS family are known to contain small molecule cofactors within their cores, and these cofactors are reportedly required for proper folding and functioning of the PAS domain within the context of the holo-protein. Specification, p.1, line 22 - p.2, line 1. However, for the vast majority of PAS domains there is no evidence for such a cofactor. In fact, structurally characterized PAS domains without bound cofactors (Amezcua et al., 2002; Erbel et al., 2003; Morais Cabral et al., 1998) show tightly packed cores with no pre-formed cavities that would suggest a cofactor or ligand binding site. Specification, p.2, lines 2-5. Since the prior work provided no evidence of cofactors for most PAS domains, and taught that those limited PAS domains having cofactors required them for proper folding, and taught that PAS domains without cofactors had tightly packed cores with no pre-formed cavities that would suggest a cofactor or ligand binding site, one skilled in the art would not have expected that such PAS domains would be rational candidates to screen for core ligand binding; in fact, the art (*supra*) teaches squarely away from such use. In my opinion one skilled in the art would have considered the claimed invention nonobvious at the time it was made.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application and any patent issuing therefrom.

Date: June 19, 2006

Professor Stephen R. Sprang